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Aerobic Soil Metabolism of Flupyrazofos

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Abstract: To elucidate the fate of flupyrazofos [*O*,*O*-diethyl *O*-(1-phenyl-3-trifluoromethyl-5-pyrazoyl)phosphorothionate] in soil, an aerobic soil metabolism study was carried out for 60 days with [^{14}C]flupyrazofos applied at a concentration of 0·38 μg g^{-1} to a loamy soil. The material balance ranged from 103·5% to 86·9% and the half-life of [^{14}C]flupyrazofos was calculated to be 13·6 days. The metabolites identified during the study were 1-phenyl-3-trifluoromethyl-5-hydroxypyrazole (PTMHP) and *O*,*O*-diethyl *O*-(1-phenyl-3-trifluoromethyl-5-pyrazoyl)phosphate (flupyrazofos oxon), with maximum levels of 9·8% and 1·6% of applied radiocarbon, respectively. Evolved [^{14}C]carbon dioxide accounted for up to 5·3% of applied radiocarbon and no volatile products were detected during the study. Non-extractable ^{14}C-residue reached 31·6% of applied material at 60 days after treatment and radiocarbon was distributed almost evenly in humin, humic acid and fulvic acid fraction. © 1998 Society of Chemical Industry

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Key words: aerobic; soil; metabolism; flupyrazofos; flupyrazofos oxon; PTMHP

1 INTRODUCTION

Flupyrazofos (KH502; *O*,*O*-diethyl *O*-(1-phenyl-3-trifluoromethyl-5-pyrazoyl)phosphorothionate; Fig. 1)† is a new phosphorothionate insecticide developed by Korea Research Institute of Chemical Technology (KRICT)[1–3] which is very effective against diamondback moth (*Plutella xylostella* (L.)) and has low mammalian toxicity [LD_{50}(rat, oral) 372–605 mg kg^{-1}].[4,5] As a contact and stomach poison, it inhibits acetylcholinesterase without showing mutagenicity, teratogenicity, delayed neurotoxicity or phytotoxicity.[5] Thermostability[6] and photostability[7] were studied and in an in-vitro metabolism study using rat liver microsomes, the corresponding phosphate (flupyrazofos oxon) and 1-phenyl-3-trifluoromethyl-5-hydroxypyrazole (PTMHP) were observed as metabolites.[8]

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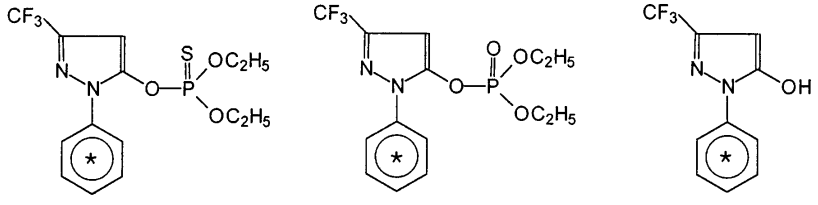
† Flupyrazofos is the proposed common name for this compound, but has not yet been finally approved by ISO.



Flupyrazofos Flupyrazofos oxon PTMHP

Fig. 1. Structures of [^{14}C]flupyrazofos, flupyrazofos oxon, and PTMHP. * Site of the ^{14}C label.

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Since a large proportion of the applied pesticide reaches the soil and a variety of transformed products can be produced by physicochemical/biochemical reactions therein,[9–11] soil metabolism studies of pesticide are very important for predicting the degradation pattern of the parent pesticide, for determining the nature and extent of the metabolites formed and for developing soil residue analysis methods for conducting terrestrial field dissipation studies.[9–13]

This paper is the first report on the soil metabolism of flupyrazofos under aerobic conditions and describes the material balance, the degradation pattern of flupyrazofos and the formation of metabolites.

2 MATERIALS AND METHODS

2.1 Test soil

Loamy soil (alluvial soil, Cheongweon Series) was obtained in Korea Research Institute of Chemical Technology, by sampling upland field soil to a depth of 10 cm. After air-drying at room temperature for 24 h, soil was sieved to remove any particles larger than 2 mm prior to being characterized (Table 1). For the experiments, moist soil at 75% of field moisture content at 1/3 bar was prepared by adding the required amount of distilled water.[12]

2.2 Radioisotopes

Radioisotope compounds were prepared for the study and their identities confirmed by co-chromatography with authentic compounds.

2.2.1 [^{14}C]*flupyrazofos*

[^{14}C]flupyrazofos, uniformly labelled in the phenyl ring, was prepared by GSF(Forschungszentrum für Umwelt und Gesundheit GmbH, Germany) in cooperation with KRICT. The purity of [^{14}C]flupyrazofos was examined by TLC/digital autoradiography and radioisotope-HPLC (RHPLC) just before the start of the experiment (radiochemical purity; >99%, specific radioactivity; 28·2 mCi mmol^{-1}).

2.2.2 Preparation of [^{14}C]*PTMHP*

To a solution of [^{14}C]flupyrazofos (355·9 mg, 11·57 mCi) in acetone (5 ml), sodium hydroxide solution (1 M, 4 ml) was added and the mixture heated at reflux for 5 h. After the reaction was complete, the reaction mixture was adjusted to pH 2 with hydrochloric acid (2 M). The acidic mixture was evaporated and acetone (10 ml) was added to the residue. The insoluble sodium chloride was removed and the organic layer evaporated to give a yellow/black solid. The crude [^{14}C]PTMHP was washed sequentially with water + hexane (1 + 1 by volume; 10 ml), cyclohexane (20 ml) and chilled dichloromethane (10 ml) and then dissolved in ethyl acetate (10 ml). The organic layer was then dried with anhydrous sodium sulfate and concentrated. The residue was further washed with anhydrous hexane (20 ml) to give an off-white solid (156·0 mg, 7·24 mCi, yield: 73·1%, radiochemical purity >99%).

2.2.3 Preparation of [^{14}C]*flupyrazofos oxon*

To a suspension of [^{14}C]PTMHP (49·7 mg, 2·64 mCi) in benzene (15 ml), potassium carbonate (0·8 g) was added and the mixture heated at reflux while removing water as an azeotrope. After the separation of water was complete, further benzene (5 ml) was distilled off and the reaction mixture was cooled to 70–75°C. Diethyl chlorophosphate (43·3 µl) was then added and the mixture heated at reflux for 18 h. After the reaction was complete, the reaction mixture was cooled to room temperature and transferred to a separating funnel with more benzene (40 ml). The benzene layer was washed with potassium carbonate solution (50 g litre^{-1}; 3 × 40 ml) and water (3 × 30 ml). The organic layer was dried with anhydrous sodium sulfate and concentrated to give a yellowish oil (101·6 mg, 2·68 mCi, yield: 128·1%, radiochemical purity: 96·4%).

2.3 Radioassay

Digital autoradiograms were obtained on a Berthold digital autoradiograph. Radioactivity of all liquid samples was quantified with Insta-gel scintillation cocktail (10 ml) using a liquid scintillation counter (LSC; Packard model Tricarb 1500). The non-extractable residue (200–400 mg) was mixed with cellulose powder (100–200 mg) and Combustaid (100–200 µl), pelletized and combusted by sample oxidizer (Packard model 307). The [^{14}C]carbon dioxide produced was absorbed in Carbo-sorb E (10 ml) and mixed with Permafluor E^{++} (10 ml) for LSC counting.

TABLE 1
Physicochemical Properties of the Test Soil

pH (1 : 5)	*Organic matter* (%)	*CEC* (*cmol kg*$^{-1}$)	*Field moisture at 1/3 bar* (%)	*Sand*	*Silt* (%)	*Clay*	*Texture* (*USDA*)
5·6	1·38	13·5	18·5	47·3	33·3	19·4	Loam

2.4 Chromatography

TLC was carried out with precoated glass plates (silica gel 60 F_{254}, 0·25 mm thickness; Merck) and developed in acetone + hexane (1 + 1 by volume). TLC plates were examined under ultraviolet light and by the digital autoradiograph after the co-chromatography with authentic standards.

Radioisotope-HPLC was performed using a thermo Separation Product (TSP) model P2000 gradient HPLC system with a C_{18} column (Inertsil, 5 μm, 4·6 × 250 mm). A two-step linear gradient [A = acetonitrile, B = acetonitrile + 10 mM NaH_2PO_4 (pH 3·16) (1 + 19 by volume): 40%A, 60%B at 0 min; 45%A, 55%B at 10 min; 70%A, 30%B at 17 min] was employed over 40 min with flow rate of 1·0 ml min^{-1}. UV detection (230 nm) and radioactivity monitoring (Berthold LB506 C-1 radioactivity monitor, 1·0 ml liquid cell) was performed using Scintillation cocktail (Readyflow III or Lumaflow II, 2 ml min^{-1}). The identity of peaks was confirmed by co-chromatography with radiolabelled reference compounds.

2.5 Extraction efficiency

Samples of soil (50 gm) were treated with [^{14}C]flupyrazofos (1·4 μCi), [^{14}C]flupyrazofos oxon (1·35 μCi) and [^{14}C]PTMHP (1·45 μCi), respectively and left for 30 min. Individual samples of treated soil were extracted with acetone (100 ml), acetone + water (1 + 1 by volume; 100 ml) or acetone + water + 36% HCl (35 + 35 + 2 by volume; 100 ml) by shaking for 1 h. In each case the extract was decanted after centrifugation at 3400 rev min^{-1} for 10 min and the same procedure was repeated three or four times until the radioactivity of the extract reached the background level. The pooled extracts were adjusted to a known volume (400–500 ml) with acetone and aliquots of extracts (1 ml) were radioassayed. The extract was then concentrated and the residue dissolved in methanol (5 ml). This solution was filtered through an 0·45 μm syringe filter (PVDF Acrodisc13, Gelman science) and 50 μl of the filtrate analysed by RHPLC.

2.6 Soil incubation

Soil (50·0 g) in a 250-ml metabolism flask was preincubated at 25(±1)°C for three weeks in the dark and [^{14}C]flupyrazofos (1·4 μCi in 100 μl of acetonitrile) was applied dropwise to soil at a concentration of 0·38 μg g^{-1}. Soil samples were then incubated for up to 60 days at 25(±1)°C. Readjustment of soil moisture content and sampling of [^{14}C]carbon dioxide and traps for volatile products were carried out once a week. Soil was extracted and the extracts analysed at 0, 3, 7, 14, 28, 60 days after treatment (DAT).

2.7 Trapping of volatiles and [^{14}C]carbon dioxide

Two polyurethane foam plugs and ethylene glycol (50 ml) were used for volatile traps and [^{14}C]carbon dioxide was trapped by two potassium hydroxide solution traps (1 M; 50 ml). Prior to sampling, the air flow rate through the system was adjusted to 10 ml min^{-1} for 30 min to transfer volatile products and [^{14}C]carbon dioxide to the traps. The foam plugs used to trap volatile products were extracted with methanol (30 ml). A sub-sample of the methanol extract (1 ml) and ethylene glycol (1 ml) from the trap was counted by LSC. To quantify evolved [^{14}C]carbon dioxide, aliquots (2 ml) of the two potassium hydroxide traps were mixed with scintillation cocktail and stored overnight at 4°C before LSC counting. Levels of [^{14}C]carbon dioxide, were confirmed by reacting the potassium hydroxide solution (10 ml) of 60 DAT sample with barium chloride solution (1 M, 10 ml). The resulting supernatant was counted after filtering of the barium [^{14}C]carbonate precipitate.

2.8 Extraction and analysis of soil

After the trapping procedure was complete, soil samples were extracted with acetone (100 ml) and analysed as in Section 2.5. In samples where more than 10% of the applied radioactivity remained in the soil after extraction, the sample was extracted with acetone + water and acetone + water + 36%HCl.

The remaining soil was air-dried, pulverized and a sub-sample combusted by sample oxidizer. Two grams of the remaining soil was then taken for the fractionation of organic matter.[14]

To determine the stability of flupyrazofos and flupyrazofos oxon in soil, soil samples were treated at a concentration of 2 μg g^{-1}, for both compounds, and incubated at 25°C for 24 h in a separate experiment. The soil was then extracted with acetone (100 ml, 50 ml) and the combined extract was analysed as in Section 2.5.

2.9 Fractionation of non-extractable soil-bound residues[14]

Two grams of non-extractable soil residues sample was extracted with sodium hydroxide solution (0·1 M, 5 ml) and the supernatant was decanted after the extract was centrifuged at 10 000 rev min^{-1} for 10 min. This procedure was repeated until the radioactivity of the extract reached the background level. Sub-samples of the precipitate were combusted to determine the radiocarbon content of the humin fraction. The extracts were combined and concentrated hydrochloric acid was added to adjust to pH 1. This mixture was centrifuged, supernatant decanted and the resulting precipitate

(humic acid fraction) was washed with hydrochloric acid (0·2 M; 5 ml). After centrifugation, supernatants (fulvic acid fraction) were combined, and the humic acid precipitate was dissolved in sodium hydroxide (0·1 M; 5 ml). Aliquots of both of the fractions were radiocounted.

3 RESULTS AND DISCUSSION

3.1 Extraction efficiency

[^{14}C]Flupyrazofos was recovered from soil in good yield (95–102%) by acetone and acetone/water extractions (Table 2). Acetone/water was a better extraction system than acetone alone for [^{14}C]flupyrazofos oxon, but [^{14}C]PTMHP was formed as a degradation product in both extraction systems. The larger amount of PTMHP present in the acetone/water extract suggested possible hydrolysis of oxon to PTMHP by water. The addition of hydrochloric acid to an aqueous acetone mixture increased the extraction efficiency for PTMHP. However, the extractability of PTMHP was still low in all three solvent systems due to the production of many degradation products. The low total recovery (57–75%) and the low PTMHP level (36–48%) in the extracts suggests that PTMHP may be quickly degraded in soil and that degradation products are strongly bound to soil.

3.2 Material balance

The material balance (Fig. 2) over the time course of the study was 86·9%–104% of applied radiocarbon. Solvent-extractable radiocarbon levels decreased gradually from 99% (0 DAT) to 50% (60 DAT) and non-extractable radiocarbon levels steadily increased as the soil aged, reaching 31·6% of applied radiocarbon at 60 DAT (Fig. 3) suggesting that binding of flupyrazofos or degradation products to soil occurred. The evolution of [^{14}C]carbon dioxide increased slowly and reached 5·3% of the applied radiocarbon, but no other volatile products were detected. From these results, it seemed that the extent of mineralization of flupyrazofos by soil microbes is low.

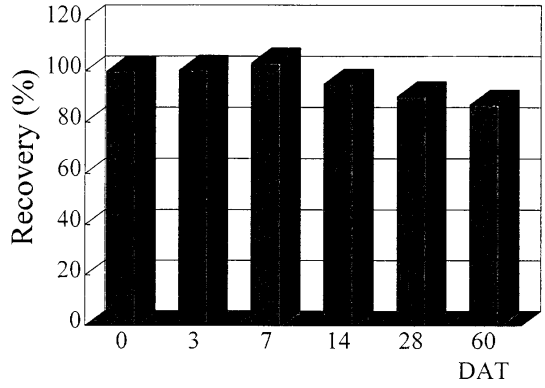

Fig. 2. Material balance of soil metabolism of [^{14}C]flupyrazofos.

To find out the distribution of radiocarbon in the non-extractable residue, further fractionation of the residue into humin, humic acid and fulvic acid was performed.[14,15] Though the humin fraction showed a

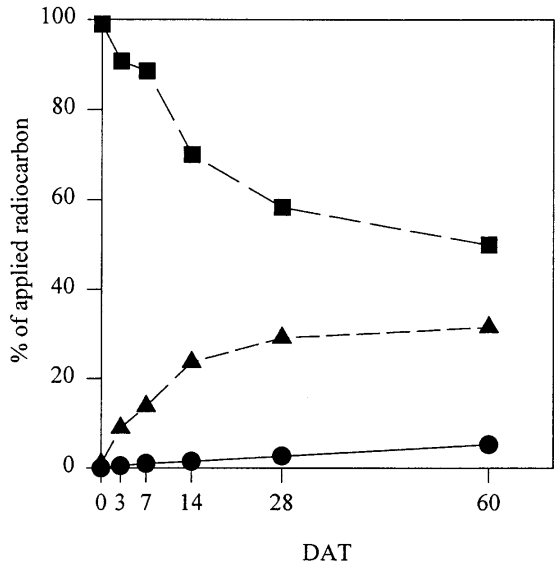

Fig. 3. Radioactivity of (■) solvent extracts, (●) [^{14}C]carbon dioxide and (▲) non-extractable residue.

TABLE 2
Extractability of [^{14}C]Flupyrazofos, Flupyrazofos Oxon and PTMHP with Different Solvent Systems

	Extracted (% of applied radiocarbon)[a]		
Compound	*Acetone*	*Acetone + water (1 + 1, by volume)*	*Acetone + water + HCl (35 + 35 + 2, by volume)*
[^{14}C]flupyrazofos	94·9 ± 2·3 (100 ± 0·0)	102 ± 1·1 (100 ± 0·0)	[b]
[^{14}C]flupyrazofos oxon	88·0 ± 0·5 (97·6 ± 0·6)	99·4 ± 1·1 (78·9 ± 2·8)	[b]
[^{14}C]PTMHP	57·6 ± 1·2 (36·1 ± 3·1)	71·0 ± (39·3 ± 2·1)	75·0 ± 1·9 (47·8 ± 2·0)

[a] Recovery in intact form in parentheses.
[b] Not performed, since the previous extractions gave good recoveries.

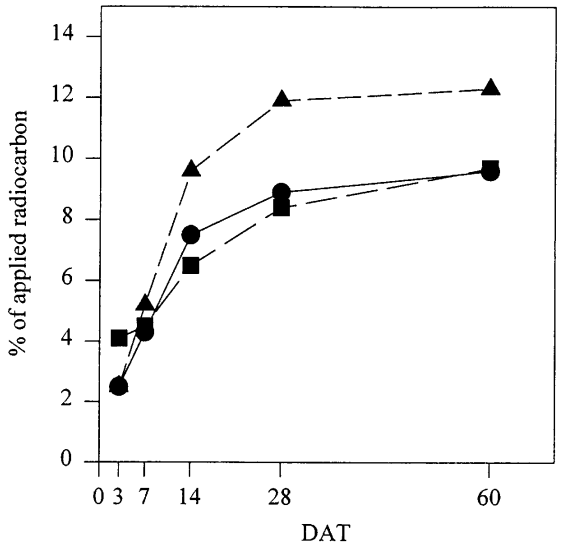

Fig. 4. Distribution of non-extractable radiocarbon in organic matter. (●) Humic acid, (■) fulvic acid, (▲) humin.

somewhat higher rate than the others, the distribution of radiocarbon was not significantly different between the three fractions, showing no characteristic binding pattern (Fig. 4).

3.3 Degradation of flupyrazofos and identification of metabolites

Using RHPLC, flupyrazofos, flupyrazofos oxon and PTMHP were separated without difficulty (Fig. 5). Flupyrazofos was observed only in the acetone extract and declined to 9·7% from 99% of applied radiocarbon after 60 days (Fig. 6), giving the calculated half-life and DT_{50} of 13·6 days ($Y = -0{\cdot}0392X + 4{\cdot}443$, $R^2 = 0{\cdot}9473$). Metabolites were identified unambiguously by *co*-chromatography and the major metabolite identified was PTMHP. It was detected only in the acetone/water/HCl extract up to a maximum level of

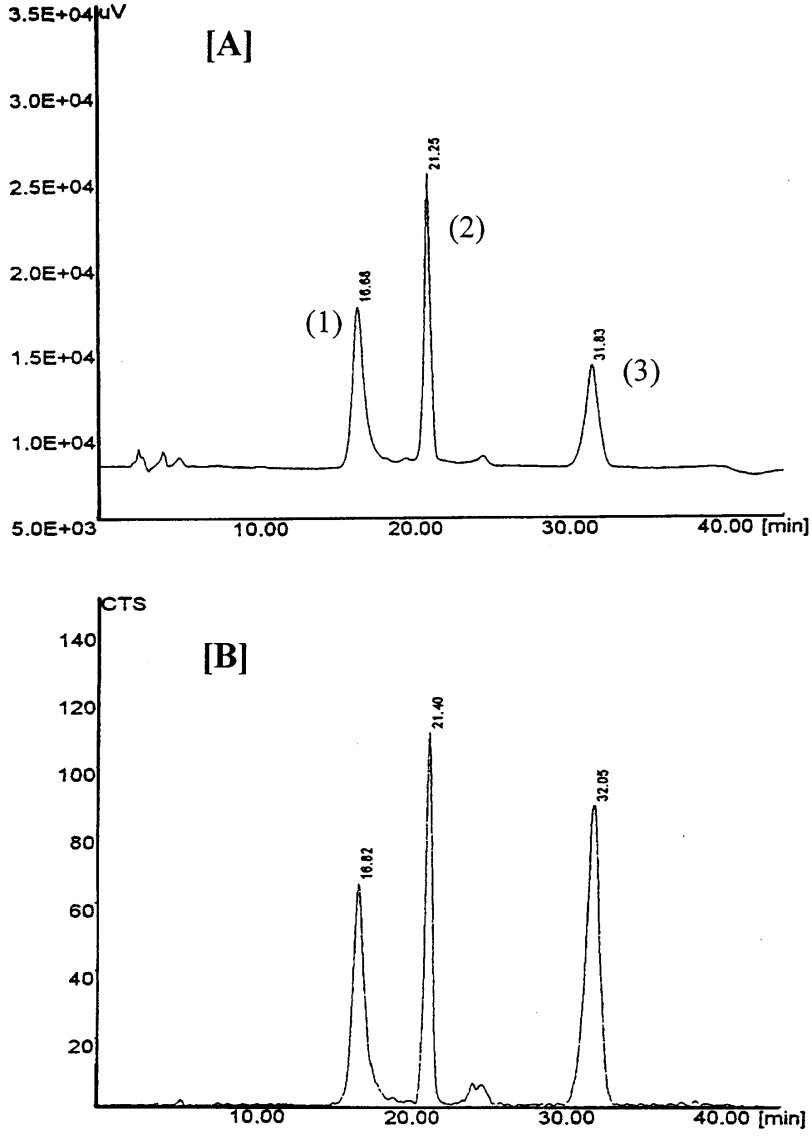

Fig. 5. RHPLC chromatograms of [^{14}C]flupyrazofos, flupyrazofos oxon and PTMHP. (A) UV trace, (B) radioactivity trace.

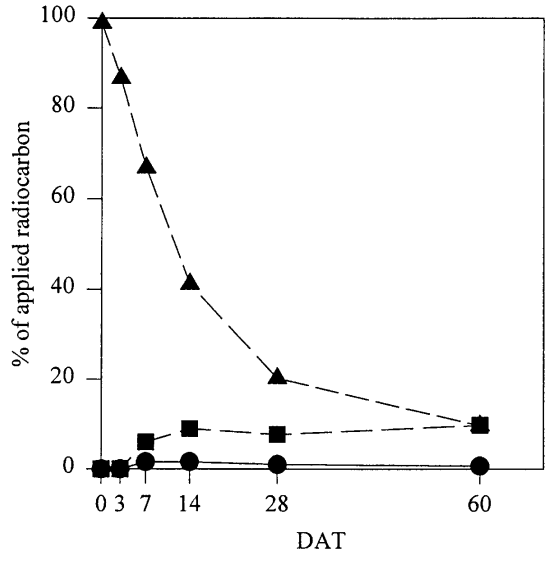

Fig. 6. (▲) Degradation of flupyrazofos and formation of (●) flupyrazofos oxon and (▲) PTMHP.

9·8% of applied radiocarbon. Flupyrazofos oxon was observed as a minor metabolite in the acetone extract at levels up to 1·6% of applied radiocarbon. These results suggests that soil metabolism of flupyrazofos follows a typical metabolic pathway[16–27] for phosphorothionates, giving the desulfurized product, flupyrazofos oxon, and the hydrolysed product, PTMHP.

In the chemical stability test in soil, 92% of flupyrazofos remained after 24 h incubation. However, all the flupyrazofos oxon disappeared, suggesting that the oxon might be hydrolysing in soil to PTMHP. Hydrolysis of flupyrazofos oxon has been observed in a microsomal study,[8] and this transformation could explain why only a small quantity of oxon was detected in the present metabolic study. Two or three unidentified polar peaks (retention time = 2·5–4·5 min) were also observed in the acetone/water and in acetone/water/HCl extracts. A possible metabolic pathway for flupyrazofos in soil under aerobic condition is proposed in Fig. 7 on the basis of the results so far.

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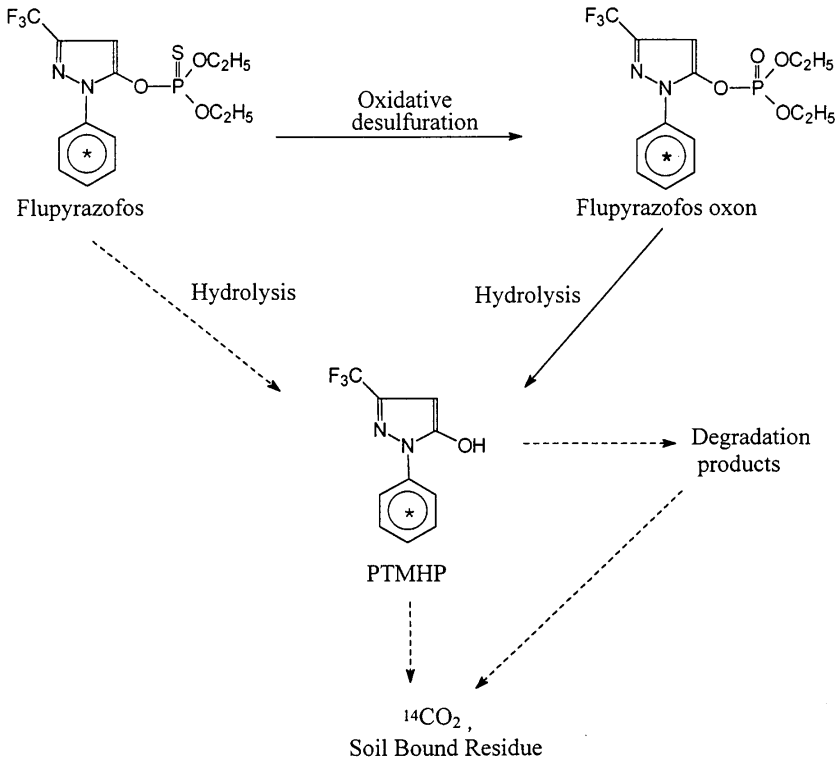

Fig. 7. Proposed metabolic pathway of [^{14}C]flupyrazofos in soil under aerobic conditions.

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